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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Method for facilitating an automated isolation of a biological target material
using magnetic beads

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Method for facilitating an automated isolation of a biological target material using magnetic beads

The present invention provides a method for isolating biological target materials, particularly nucleic acids, such as DNA or RNA or hybrid molecules of DNA and RNA, from other substances in a medium using silica magnetic particles. The method of the present invention involves forming a complex of the silica magnetic particles and the biological target material in a mixture of the particles and the medium, comprising a salt and a volume excluding agent, separating the complex from the mixture using external magnetic force, and eluting the biological target material from the complex, in a preferred embodiment in an automated process.

The present invention relates to methods for separating or isolating a biological target material from other substances in a medium to produce an isolated material of sufficient purity for further processing or analysis. The present invention particularly relates to methods for separating or isolating biological target materials using magnetically responsive particles capable of reversibly binding the material. The present invention more specifically relates to methods for separating or isolating biological target materials using at least one magnetically responsive particle comprising silica or a silica derivative such as silica gel which reversibly binds the biological target material thereof. The present invention even more specifically relates to methods for separating or isolating biological target materials as mentioned above in an automated process.

Obtaining DNA or RNA sufficiently free of contaminants for molecular biological applications is complicated by the complex systems in which the DNA or RNA is typically found. These systems, e.g. plant seed samples, leaf samples, tissue samples, cells from body fluids such as blood, lymph, milk, urine, feces, semen, or the like, cultured cells, agarose or polyacrylamide gels, or solutions in which target nucleic acid amplification has been carried out, typically include significant quantities of contaminants from which the DNA or RNA of interest must be isolated before being used in a molecular biological procedure.

Conventional protocols for obtaining DNA or RNA from cells are described in the literature (e.g. Chapter 2 (DNA) and Chapter 4 (RNA) of F. Ausubel et al., eds., Current Protocols in Molecular Biology, Wiley-Interscience, New York, 1993). Conventional DNA isolation protocols generally entail suspending the cells in a 5 solution and using enzymes and/or chemicals, gently to lyse the cells, thereby releasing the DNA contained within the cells into the resulting lysate solution. For isolation of RNA, the conventional lysis and solubilization procedures include measures for inhibition of ribonucleases and contaminants to be separated from the RNA including DNA.

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Silica materials, including glass particles, such as glass powder, silica particles, and glass microfibers prepared by grinding glass fiber filter papers, and including diatomaceous earth, have been employed in combination with aqueous solutions of chaotropic salts to separate DNA from other substances and render the DNA suitable 15 for use in molecular biological procedures (e.g. US 5,075,430; Marko et al., Anal. Biochem. 121, 382-387, 1982; Vogelstein et al., Proc. Natl. Acad. Sci. (USA) 76, 615-619, 1979; Boom et al., J. Clin. Microbiol. 28, 495-503, 1990; Chen and Thomas, Anal. Biochem. 101, 339-341, 1980).

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Glass particles, silica particles, silica gel, and mixtures of the above have been configured in various different forms to produce matrices capable of reversibly binding nucleic acid materials when placed in contact with a medium containing such materials in the presence of chaotropic agents. Such matrices are designed to remain bound to the nucleic acid material while the matrix is exposed to an external 25 force such as centrifugation or vacuum filtration to separate the matrix and nucleic acid material bound thereto from the remaining media components. The nucleic acid material is then eluted from the matrix by exposing the matrix to an elution solution, such as water or an elution buffer. Numerous commercial sources offer silica-based matrices designed for use in centrifugation and/or filtration isolation systems (e.g. the 30 QiaPrep™ line of DNA isolation systems from QIAGEN, Hilden, Germany).

Magnetically responsive particles (referred to as 'magnetic particles') have conventionally been used to isolate and purify polypeptide molecules such as proteins or antibodies. In recent years, however, magnetic particles and methods for

using magnetic particles have been developed for the isolation of nucleic acid materials. Several different types of magnetic particles designed for use in nucleic acid isolation are described in the literature, and many of those types of particles are available from commercial sources. Such magnetic particles generally fall into either 5 of two categories, those designed to reversibly bind nucleic acid materials directly, and those designed to do so through at least one intermediary substance. The intermediary substance is referred to herein as a 'label'.

10 The magnetic particles designed to bind nucleic acid materials indirectly are generally used to isolate a specific nucleic acid material, such as mRNA, according to the following basic isolation procedure. First, a medium containing a nucleic acid material is placed in contact with a label capable of binding to the nucleic acid material of interest. For example, one such commonly employed label, biotinylated oligonucleotide deoxythymidine (oligo-dT), forms hydrogen bonds with the poly- 15 adenosine tails of mRNA molecules in a medium. Each label so employed is designed to bind with a magnetically responsive particle, when placed into contact with the particle under the proper binding conditions. For example, the biotin end of a biotinylated oligo-dT/mRNA complex is capable of binding to streptavidin moieties on the surface of a streptavidin coated magnetically responsive particle. Several 20 different commercial sources are available for streptavidin magnetic particles and reagents designed to be used in mRNA isolation using biotinylated oligo-dT as described above (e.g. the ProActive™ line of streptavidin coated microsphere particles from Bangs Laboratories, Carmel, USA).

25 A few types of magnetic particles have also been developed for use in the direct binding and isolation of biological materials, particularly nucleic acids. One such particle type is a magnetically responsive siliceous-oxide coated bead (e.g. MagAttract® magnetic beads, QIAGEN, Hilden, Germany; MagneSil™ magnetic beads, Promega, Madison, USA). Nucleic acids adhere to these particles in the 30 presence of a chaotropic salt, e.g. guanidine hydrochloride or guanidine isothiocyanate, alone or in combination with a binding additive like an alcohol, e.g. ethanol. After separation of the particles, desorption of the bound nucleic acids is achieved easily by incubation of the beads in a buffer with low ionic strength.

Several different methods of automated separation of magnetic beads are known from the art. The first method is to insert a magnetic or magnetizable device into the medium containing the magnetic beads, binding the magnetic beads to the magnetic or magnetizable device, and remove the magnetic or magnetizable device. The 5 second method is to remove the medium containing the magnetic particles with a pipette and to bring a magnetic or magnetizable device into spatial proximity of the pipette tip. The magnetic beads are kept back in the pipette tip when the medium is removed from the pipette tip. These two methods require special technical means, e.g. a robot constructed especially for one of those methods. A more general 10 principal in removing magnetic beads is to bring a magnetic or magnetizable device into spatial proximity to the container containing the medium and the magnetic beads. The magnetic beads bind to the container wall and the medium can be removed without magnetic beads.

15 Methods as known from the state of the art, particularly those methods using alcohols as binding additives, show the substantial disadvantage of massive clustering of the magnetic particles after binding of nucleic acids and prior to the removal of the particles. This impedes the use of such a method in an automated process due to potential physical interferences of the clustered particles with, e.g., 20 pipette tips which may results in only partial removal of the magnetic beads from the medium and/or disturbance of subsequent automated steps.

The problem underlying the present invention is that methods as known from the state of the art are utilizable in automated processes only on a limited scale due to 25 the potentially massive clustering of the magnetic beads. Thus, there is need in the art to accomplish such methods capable of being automated. Therefore, the method according to this invention is from particular interest in high throughput analyses.

The present invention discloses a method for facilitating an automated isolation of 30 biological entities, particularly nucleic acids, using magnetically responsive particles capable of rapidly and efficiently isolating such entities, wherein no substantial clustering of the magnetic particles occurs and performance of the method in an automated process is undisturbed.

The problem is solved by utilizing volume excluding agents, e.g. polyalkylene glycols, dextran sulfate etc., at relatively low concentrations during the isolation procedure to avoid clustering of the magnetic particles. Briefly, the present invention comprises a method for isolating a biological target material from other materials in a medium by:

- 5 1) providing a medium including the biological target material;
- 2) providing magnetic particles;
- 3) forming a complex of the magnetic particles and the biological target material by combining the magnetic particles and the medium comprising a salt and a volume excluding agent;
- 10 4) removing the complex from the medium by application of an external magnetic field and
- 5) separating the biological target material from the complex by eluting the biological target material, whereby the isolated biological target material is obtained.

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The biological target material isolated according to the method of this invention is preferably a nucleic acid, more preferably DNA. The magnetic particles according to this invention are preferably silica magnetic particles, more preferably the particles are siliceous-oxide coated particles.

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A preferred practice of the method of the present invention comprises the following steps. First, a mixture is formed comprising the medium (comprising a salt and a volume excluding agent) and the silica magnetic particles. Second, the biological target material is adhered to the silica magnetic particles in the mixture. Third, the silica magnetic particles are removed from the mixture using an external force, most preferably using a magnetic force, and Fourth, the biological target material adhered to the silica magnetic particle is substantially eluted by contacting the particle with an elution solution.

25 30 In a further aspect, the present invention comprises a kit for isolating a biological target material from a medium containing the same, the kit comprising an aliquot of magnetic particles, preferably silica magnetic particles and most preferably siliceous-oxide coated magnetic particles suspended in an aqueous solution in a first container. Optionally, the kit may include other components needed to isolate a

biological target material from a medium containing the same according to the methods of the present invention. Preferably, the kit comprises an aliquot of magnetic particles, more preferably silica magnetic particles and most preferably siliceous-oxide coated magnetic particles, a chaotropic salt or a chaotropic salt solution, and a 5 volume excluding agent.

As used herein, the term 'magnetic' encompasses magnetic materials, such as ferromagnetic, ferrimagnetic, paramagnetic or superparamagnetic materials.

10 The term 'silica magnetic particle' refers to a magnetic particle comprised of silica in the form of silica gel, siliceous oxide, solid silica such as glass or diatomaceous earth, or a mixture of two or more of the above. The term 'silica gel' as used herein refers to chromatography grade silica gel, a substance which is commercially available from a number of different sources. Silica gel is most commonly prepared 15 by acidifying a solution containing silicate, e.g. sodium silicate, to a pH of less than 10 or 11 and then allowing the acidified solution to gel (e.g. silica preparation discussion in Kurt-Othmer Encyclopedia of Chemical Technology, Vol. 6, 4th ed., Mary Howe-Grant, ed., John Wiley & Sons, pub., 1993, pp. 773-775).

20 The term 'glass particles' as used herein means particles of crystalline silicas (e.g., α -quartz, vitreous silica), even though crystalline silicas are not formally 'glasses' because they are not amorphous, or particles of glass made primarily of silica.

25 The term 'siliceous-oxide coated magnetic particle' is used herein to refer to the most preferred form of silica magnetic particle used in the methods and kits of the present invention. The siliceous-oxide coated magnetic particle is comprised of siliceous oxide coating a core of at least one particle of ferrimagnetic, ferromagnetic, superparamagnetic or paramagnetic material. The siliceous-oxide coated magnetic particle used in the present method and kits also has an adsorptive surface of 30 hydrous siliceous oxide. Target nucleic acid material, such as DNA or RNA, adhere to the adsorptive surface of the particle while other material, particularly deleterious contaminants such as nucleases, do not adhere to or co-elute from the particle with the nucleic acid materials.

The term 'volume excluding agent' as used herein refers to non-ionic substances which are strongly hydratable. In a preferred embodiment, these volume excluding agents are selected from the group of polyalkylene glycols, cyclodextrin, carrageen, dextran sulfate, cellulose, amylose and/or polyvinyl alcohol. The above mentioned

5 polyalkylene glycols include but are not limited to polyethylene glycol and/or polypropylene glycol. In the most preferred embodiment the volume excluding agent is polyethylene glycol. Surprisingly, volume excluding agents are able to inhibit the clustering of the magnetic particles at relatively low concentrations.

10 The present invention provides convenient and efficient means for isolating biological target material of interest from a variety of different media. One aspect of the present method described briefly above, wherein magnetic force is used to remove the particles from the media, offers significant advantages over conventional isolation methods wherein a biological target material is reversibly bound to other silica 15 material. Specifically, the magnetic removal step of the method substitutes for vacuum filtration or centrifugation steps required in conventional silica binding and elution isolation methods.

20 A preferred aspect of the present invention is the facilitation of automation of isolation of biological target material of interest from a variety of different media. Methods as known from the state of the art, particularly those methods using alcohols as binding additives, show the substantial disadvantage of massive clustering of the magnetic particles prior to the removal of the particles, especially when genomic DNA is adhered to the particles. This impedes the use of such a method in an automated 25 process due to potential physical interferences of the clustered particles with, e.g., pipette tips which may results in only partial removal of the magnetic beads from the medium and/or disturbance of subsequent automated steps. For example, isolation of nucleic acids from a medium in an automated process, e.g. using a BioRobot® 3000 (QIAGEN, Hilden, Germany), is often disturbed due to complications in handling of the clustered beads. The utilization of volume excluding agents in relatively low concentrations according to the present invention surprisingly inhibits the clustering of the particles and allows for the automation of the process without physical interferences as mentioned above. Therefore, the method according to this 30 invention is from particular interest in high throughput analyses.

The biological target material isolated using the method of the present invention is sufficiently free of contaminating material for additional processing or analysis using standard molecular biology techniques. Applications of the present methods to 5 isolate various different biological target materials from a variety of different media will become apparent from the detailed description of the invention below.

The biological target material isolated using the methods of the present invention is preferably a nucleic acid or a protein, more preferably a nucleic acid material such as 10 RNA, DNA, PNA, or hybrid thereof. When the biological target material isolated using the present methods is a nucleic acid, it is preferably DNA or RNA including but not limited to total DNA, genomic DNA, plasmid DNA, DNA fragments produced from restriction enzyme digestion, amplified DNA produced by an amplification reaction such as the polymerase chain reaction (PCR), single-stranded DNA, mRNA, or total 15 RNA.

Since nucleic acids are the most preferred biological target material isolated using the methods of the present invention, most of the detailed description of the invention below describes this preferred aspect of the present invention. However, the detailed 20 description of this particular aspect of the present invention is not intended to limit the scope of the invention. The present disclosure provides sufficient guidance to enable one of ordinary skill in the art of the present invention to use the methods of the present invention to isolate biological target materials other than nucleic acid materials, e.g. proteins or antibodies.

25 The present methods of isolating biological target material can be practiced using any magnetic particle, but silica magnetic particles are preferred. Even more preferably, the methods are practiced using any form of siliceous-oxide coated magnetic particles, e.g. MagAttract® magnetic beads (QIAGEN, Hilden, Germany). 30 The silica magnetic particles used in the methods of this invention may be any one of a number of different sizes. Smaller silica magnetic particles provide more surface area (one per weight unit basis) for adsorption, but smaller particles are limited in the amount of magnetic material which can be incorporated into such particles compared to larger particles.

The biological target material isolated using the method of the present invention can be obtained from eukaryotic or prokaryotic cells in culture or from cells taken or obtained from tissues, multicellular organisms including animals and plants; body fluids such as blood, lymph, urine, feces, or semen; embryos or fetuses; food stuffs; cosmetics; or any other source of cells. Some biological target materials, such as certain species of DNA or RNA are isolated according to the present method from the DNA or RNA of organelles, viruses, phages, plasmids, viroids or the like that infect cells. Cells will be lysed and the lysate usually processed in various ways familiar to those skilled in the art to obtain an aqueous solution of DNA or RNA, to which the separation or isolation methods of the invention are applied. The DNA or RNA, in such a solution, will typically be found with other components, such as proteins, RNAs (in the case of DNA separation), DNAs (in the case of RNA separation), or other types of components.

15 Regardless of the nature of the source of such material, the biological target material to be isolated in the present methods is provided in a medium comprising the biological target material and other species. The biological target material must be present in the medium in a form in which it is available to adhere to the silica magnetic particles in the first step of the method. When the nucleic acid material is contained inside a cell, the cell walls or cell membrane can make the material unavailable for adhesion to the particles. Even if such cells are lysed or sufficiently disrupted to cause the nucleic acid material contained therein to be released into the surrounding solution, cellular debris in the solution could interfere with the adhesion of the nucleic acid material to the silica magnetic particles. Therefore, in cases where the nucleic acid material to be isolated using the methods of the present invention is contained within a cell, the cell is preferably first processed by lysing or disrupting the cell to produce a lysate, and more preferably additionally processed by clearing the lysate of cellular debris (e.g. by centrifugation or vacuum filtration) likely to interfere with adhesion of the nucleic acid material to silica magnetic particles when provided as the medium in the methods of the present invention.

Any one of a number of different known methods for lysing or disrupting cells to release nucleic acid materials contained therein are suitable for use in producing a

medium from cells for use in the present invention. The method chosen to release the nucleic acid material from a cell will depend upon the nature of the cell containing the material. For example, in order to cause a cell with a relatively hard cell wall, such as a fungus cell or a plant cell, to release the nucleic acid material contained therein one may need to use harsh treatments such as potent proteases and mechanical shearing with a bead mill or a homogenizer, or disruption with sound waves using a sonicator. Contrastingly, nucleic acid material can be readily released from cells with lipid bilayer membranes such as *E. coli* bacteria or animal blood cells merely by suspending such cells in an aqueous solution and adding a detergent to the solution.

Once the nucleic acid material is released from cells lysed or disrupted as described above, cellular debris likely to interfere with the adhesion of the nucleic acid material to silica magnetic particles can be removed using a number of different known techniques or combination of techniques. The solution of lysed or disrupted cells is preferably centrifuged to remove particulate cell debris. Optionally, the supernatant is subsequently further processed by adding a second solution to the supernatant which causes a precipitate of additional other material to form, and then removing the precipitate from the resulting solution by centrifugation.

The medium provided in the first step of the method of this invention does not have to contain nucleic acid material released directly from cells. The nucleic acid material can be the product of an amplification reaction, such as amplified DNA produced using the polymerase chain reaction (PCR). The nucleic acid material can also be in the form of fragments of DNA produced by digesting DNA with a restriction enzyme. The medium can also be in the form of a mixture of melted or enzymatically digested electrophoresis gel and nucleic acid material.

The magnetic particles provided in the second step of the methods of the present invention preferably have the capacity to form a complex with the nucleic acid material in the medium by reversibly binding nucleic acid material. The magnetic particles provided in the second step of the methods of the present invention are more preferably silica magnetic particles and most preferably siliceous-oxide coated magnetic particles.

A complex of the silica magnetic particles and the biological target material is formed in the third step, preferably by exposing the particles to the medium containing the target material under conditions designed to promote the formation of the complex.

5 According to the invention, the complex is formed in a mixture of the silica magnetic particles and the medium comprising a salt and a volume excluding agent.

Volume Excluding agents are non-ionic substances which are strongly hydratable.

10 According to the invention, these volume excluding agents are preferably selected from the group of polyalkylene glycols, cyclodextrin, carrageen, dextran sulfate, cellulose, amylose and/or polyvinyl alcohol. The above mentioned polyalkylene glycols include but are not restricted to polyethylene glycol and/or polypropylene glycol. In the most preferred embodiment the volume excluding agent is polyethylene glycol. Surprisingly, volume excluding agents are able to inhibit the clustering of the 15 magnetic particles at relatively low concentrations which allows for a trouble-free automated process, e.g. no blockage of pipette tips due to clustered magnetic particles occurs. Therefore, the method according to this invention is from particular interest in high throughput analyses.

20 To solve the problem of clustering of the magnetic beads the volume excluding agents are utilized at a relatively low concentrations. The final concentration of the volume excluding agent, which means the concentration in the step of forming a complex of the magnetic particles and the biological target material by combining the magnetic particles and the medium comprising a salt and a volume excluding agent, 25 in the method according to the invention is adjusted to between about 2% (w/v) and 7% (w/v). Preferably, the final concentration is adjusted to about 3% (w/v) to 6% (w/v), more preferably to about 3% (w/v) and 5% (w/v), and most preferably to about 3% (w/v) to 4.8% (w/v).

30 In a preferred embodiment of the present invention the volume excluding agent is polyethylene glycol with a molecular weight of between 4000 and 12000, more preferably polyethylene glycol with a molecular weight of between 6000 and 10000, and most preferably polyethylene glycol with a molecular weight of about 8000.

The term 'salt' is used herein refers to chaotropic and non-chaotropic salts. Chaotropic salts are salts of chaotropic ions according to the 'Hoffmeister-Reihe'. Such salts are highly soluble in aqueous solutions. The chaotropic ions provided by such salts, at sufficiently high concentration in aqueous solutions of proteins or nucleic acids, cause proteins to unfold, nucleic acids to lose secondary structure or, in the case of double-stranded nucleic acids, melt (i.e. strand-separate). It is thought that chaotropic ions have these effects because they disrupt hydrogen-bonding networks that exist in liquid water and thereby make denatured proteins and denatured nucleic acids thermodynamically more stable than their correctly folded or structured counterparts. Chaotropic ions include guanidine, iodide, perchlorate and trichloroacetate. Preferred in the present invention is the guanidine ion. Chaotropic salts include guanidine isothiocyanate, guanidine thiocyanate, guanidine hydrochloride, sodium iodide, potassium iodide, lithium chloride, sodium perchlorate and/or sodium trichloroacetate. Preferred in the present invention are the guanidine salts, i.e. guanidine isothiocyanate, guanidine thiocyanate and/or guanidine hydrochloride. Non-chaotropic salts are salts of non-chaotropic ions according to the 'Hoffmeister-Reihe'. Non-chaotropic salts according to the invention include preferably sodium chloride, potassium chloride, ammonium chloride, calcium chloride and/or magnesium chloride.

20 The salt concentration in the mixture formed in the third step of the present method, which means the concentration in the step of forming a complex of the magnetic particles and the biological target material by combining the magnetic particles and the medium comprising a salt and a volume excluding agent, is preferably between 25 about 0.1 M and 10 M. With any salt used in the invention, it is desirable that the concentration of the salt, in any of the solutions in which the salt is employed in carrying out the invention, remain below the solubility of the salt in the solution under all of the conditions to which the solution is subjected in carrying out the invention. The concentration of the salt in the mixture must be sufficiently high to cause the 30 biological target material to adhere to the silica magnetic particles in the mixture, but not so high as to substantially denature, to degrade, or to cause the target material to precipitate out of the mixture. Proteins and large molecules of double-stranded DNA, such as chromosomal DNA, are stable at chaotropic salt concentrations between 0.5 M and 2 M, but are known to precipitate out of solution at chaotropic salt

concentrations above about 2 M (e.g. US. 5,346,994, column 2, lines 56-63). Contrastingly, RNA and smaller molecules of DNA such as plasmid DNA, restriction or PCR fragments of chromosomal DNA, or single-stranded DNA remain undegraded and in solution at chaotropic salt concentrations between 2 M and 5 M, which is well known to those skilled in the art. Thus, the salt concentration in a method according to the invention is dependent on the area of application and is apparent to those skilled in the art or is readily determinable.

In a practice of the method according to the present invention, the mixture formed as described above is incubated until at least some of the nucleic acid material is adhered to the silica magnetic particle to form a complex. This incubation step is carried out at a temperature of at least 0°C, preferably at least 4°C, and more preferably at least 20°C, provided that the incubation temperature is no more than 65°C. The incubation step must be carried out at a temperature below the temperature at which the silica magnetic particles begin to loose their capacity to reversibly bind the nucleic acid material. The incubation step is most preferably carried out at about room temperature (i.e. at about 25°C). The incubation is carried out over a sufficient period of time, allowing the target material to adhere adequately to the magnetic beads, i.e. at least 30 seconds.

The complex is removed from the mixture using a magnetic field. Other forms of external force in addition to the magnetic field can also be used to isolate the biological target substance according to the methods of the present invention after the initial removal step. Suitable additional forms of external force include, but are not limited to, gravity filtration, vacuum filtration and centrifugation.

In a preferred aspect of the methods of the present invention, the complex removed from the medium in the fourth step as described above is washed at least once by being rinsed in a washing buffer. The washing buffer used in this preferred additional step of the method preferably comprises a solution capable of removing contaminants from the silica magnetic particles. Any suitable washing buffer known from the state of the art can be used in the present invention. The washing buffer preferably comprises a salt and a solvent or a solvent alone, wherein the solvent is preferably an alcohol. The concentration of salt in the wash solution is sufficiently

high to ensure the nucleic acid material is not eluted from the silica magnetic particles during the washing step(s).

5 The complex is preferably washed after removal from the medium by resuspending the complex in the washing buffer. The complex is preferably removed from the washing buffer after the first wash, and more preferably is washed at least once more, using fresh washing buffer for every wash step.

10 Fifth, and finally, the nucleic acid material is eluted from the silica magnetic particle by exposing the complex to an elution buffer. The elution buffer is preferably an aqueous solution of low ionic strength at about a pH at which the nucleic acid material is stable and substantially intact. The low ionic strength of the preferred forms of the elution buffer described above ensures the nucleic acid material is released from the particle. Elution buffers suitable for use in the methods of this 15 invention will be readily apparent to one skilled in this art.

20 The nucleic acid material eluted from the complex in the elution step of the method is preferably separated from the silica magnetic particles and complexes remaining in the elution mixture by external force, such as centrifugation or a magnetic field.

25 The nucleic acid material eluted using the method of the present invention is suitable, without further isolation, for analysis or further processing by molecular biological procedures. The eluted nucleic acid can be analyzed by, for example, sequencing, restriction analysis, or nucleic acid probe hybridization. Thus, the methods of the invention can be applied as part of methods, based on analysis of DNA or RNA, for, among other things, genotyping, diagnosing diseases; identifying pathogens; testing foods, cosmetics, blood or blood products, or other products for contamination by pathogens; forensic testing; paternity testing; and sex identification of fetuses or embryos.

30 The eluted DNA or RNA provided by the method of the invention can be processed by any of various exonucleases and endonucleases that catalyze reactions with DNA or RNA, respectively, and, in the case of DNA, can be digested with restriction enzymes, which cut at restriction sites present in the DNA. Restriction fragments from

the eluted DNA can be ligated into vectors and transformed into suitable hosts for cloning or expression. Segments of the eluted DNA or RNA can be amplified by any of the various methods known in the art for amplifying target nucleic acid segments. If eluted DNA is a plasmid or another type of autonomously replicating DNA, it can be 5 transformed into a suitable host for cloning or for expression of genes on the DNA which are capable of being expressed in the transformed host.

The following, non-limiting examples teach some embodiments of the invention. In 10 the examples, and elsewhere in the specification and claims, volumes and concentrations are at room temperature unless specified otherwise. Only the most preferred form of the magnetic silica particles was used in each of the examples below, i.e. siliceous-oxide coated magnetic particles. However, one skilled in the art of the present invention will be able to use the teachings of the present disclosure to 15 select and use forms of the magnetic particles other than the siliceous-oxide coated magnetic particles whose use is illustrated in the aspects of the methods of the present invention demonstrated in the examples below.

20 Example 1: Automated isolation of genomic DNA from wheat leafs

The isolation was processed on a BioRobot® 3000 in combination with a BioRobot® 25 RapidPlate™ (both QIAGEN, Hilden, Germany). 50 mg of wheat leaves were frozen in liquid nitrogen and homogenized in a MixerMill MM 300 (Retsch, Haan, Germany). 400 µl of buffer A (3,5 M guanidine Isothiocyanate; 25 mM sodium citrate; pH 7,0) were added, thoroughly vortexed, and subsequently centrifuged at 6000xg for 5 minutes. 200 µl supernatant were transferred into a flat bottom microwell plate. 65 µl of 20 % (w/v) polyethylene glycol 8000 and 20 µl MagAttract® magnetic beads (150 mg / ml; QIAGEN, Hilden, Germany) were added. The solution was mixed and 30 incubated for 5 minutes at room temperature. Magnetic separation was applied and the supernatant was removed. Subsequently, beads were washed by resuspending in 200 µl buffer B (1,071 M guanidine hydrochloride; 107,1 mM potassium acetate; 50% (v/v) isopropanol; 100 ng/ml RNase A) followed by applying magnetic separation and removing of supernatant. The washing step as described was

repeated twice with 100% ethanol. Afterwards, the beads were dried 5 minutes at room temperature. For elution of genomic DNA the beads were resuspended in 100 µl of buffer C (10 mM Tris / HCl; pH 8,5) and incubated for 5 minutes at room temperature.

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6 to 10 µg of genomic DNA were separated per 50 mg of wheat leaves using this protocol. No problems regarding bead handling were observed during the process, i.e. no apparent bead aggregation was observed. In comparison, in an identical protocol with the exception of adding 65 µl polyethylene glycol 8000 but 225 µl 100% ethanol, in 5 to 10 wells of a 96 well plate the beads aggregated and were partially sucked into the pipette tip or the beads occluded the pipette tip and subsequent automated steps were massively disturbed.

15

Example 2: Automated isolation of genomic DNA from conifer needles

The isolation was processed on a BioRobot[®] 3000 in combination with a BioRobot[®] RapidPlate[™] (both QIAGEN, Hilden, Germany). 2 x 2 cm of conifer needles were frozen in liquid nitrogen and homogenized in a MixerMill (Retsch, Haan, Germany). 400 µl of buffer D (1,4% (w/v) sodium dodecyl sulfate; 50 mM ethylene diamine tetra acetic acid (EDTA); 500 mM sodium chloride; 2% (w/v) polyvinylpyrrolidone; 100mM sodium acetate; pH 5,5) were added, thoroughly vortexed, and subsequently centrifuged at 6000xg for 5 minutes. The further preparation of the probes was identical to the preparation described in Example 1.

No problems regarding bead handling were observed during the process, i.e. no apparent bead aggregation was observed. In comparison, in an identical protocol with the exception of adding 65 µl polyethylene glycol 8000 but 225 µl 100% ethanol, in 5 to 10 wells of a 96 well plate the beads aggregated and were partially sucked into the pipette tip or the beads occluded the pipette tip and subsequent automated steps were massively disturbed.

Example 3: Isolation of genomic DNA from wheat using different volume excluding agents

30 mg of wheat leaves were homogenized as described above, wherein the added
5 volume excluding agent was not 65 µl 20% (w/v) polyethylene glycol 8000 but 50 µl
25% (w/v) dextran sulfate. No problems regarding bead handling were observed
during the process, i.e. no apparent bead aggregation was observed.

Equal observations were made with different volume excluding agents applying the
10 same protocol. 50 µl 20% (w/v) cyclodextrin, 50 µl 20% (w/v) amylose and 50 µl 20%
(w/v) cellulose were used as additives with an identical result.

Claims:

1. A method for isolating a biological target material from other material in a medium comprising the steps of:
 - 5 a) providing a medium including the biological target material;
 - b) providing magnetic particles capable of reversibly binding the biological target material;
 - c) forming a complex of the magnetic particles and the biological target material by combining the magnetic particles and the medium in the presence of a salt and a volume excluding agent;
 - 10 d) removing the complex from the medium by application of an external magnetic field; and
 - e) separating the biological target material from the complex by eluting the biological target material, whereby the isolated biological target material is obtained.
2. A method according to claim 1, wherein the biological material isolated according to the method consists of a nucleic acid.
- 20 3. A method according to claim 2, wherein the nucleic acid is DNA.
4. A method according to claim 1, wherein the magnetic particles provided in step b) of the method are silica magnetic particles.
- 25 5. A method according to claim 4, wherein the silica magnetic particles are siliceous-oxide coated magnetic particles.
6. A method according to claim 1, wherein the salt is a chaotropic salt.
- 30 7. A method according to claim 6, wherein the chaotropic salt is selected from the group of guanidine isothiocyanate, guanidine thiocyanate, guanidine hydrochloride, sodium iodide, potassium iodide, lithium chloride, sodium perchlorate and/or sodium trichloroacetate.

8. A method according to claim 7, wherein the chaotropic salt is selected from the group of guanidine isothiocyanate, guanidine thiocyanate and/or guanidine hydrochloride.
- 5 9. A method according to claim 1, wherein the salt is a non-chaotropic salt.
- 10 10. A method according to claim 9, wherein the non-chaotropic salt is selected from the group of sodium chloride, potassium chloride, ammonium chloride, calcium chloride or magnesium chloride.
11. A method according to claims 1 and 6 to 10, wherein the concentration of the salt in the mixture formed in step (c) of the method is between 0,1 M and 10 M.
12. A method according to claim 1, wherein the volume excluding agent is selected from the group of polyalkylene glycol, cyclodextrin, carrageen, dextran sulfate, cellulose, amylose and/or polyvinyl alcohol.
13. A method according to claim 12, wherein the polyalkylene glycol is polyethylene glycol and/or polypropylene glycol.
- 20 14. A method according to claims 12 to 13, wherein the volume excluding agent is polyethylene glycol.
15. A method according to claim 14, wherein the polyethylene glycol has a molecular weight of between 4000 and 12000.
- 25 16. A method according to claim 15, wherein the polyethylene glycol has a molecular weight of between 6000 and 10000.
- 30 17. A method according to claim 16, wherein the polyethylene glycol has a molecular weight of about 8000.

18. A method according to claims 1 and 12 to 17, wherein the concentration of the volume excluding agent in the mixture formed in step c) of the method is adjusted to between about 2% (w/v) and 7% (w/v).
- 5 19. A method according to claim 18, wherein the concentration of the volume excluding agent in the mixture formed in step c) of the method is adjusted to between about 3% (w/v) and 6% (w/v).
- 10 20. A method according to claim 19, wherein the concentration of the volume excluding agent in the mixture formed in step c) of the method is adjusted to between about 3% (w/v) and 5% (w/v).
- 15 21. A method according to claim 20, wherein the concentration of the volume excluding agent in the mixture formed in step c) of the method is adjusted to between about 3% (w/v) and 4,8% (w/v).
22. A method according to claim 1, wherein the biological target material is adhered to the silica magnetic particle in step c) by incubating the mixture.
- 20 23. A method according to claim 22, wherein the biological target material is adhered to the silica magnetic particle in step c) by incubating the mixture at room temperature for at least 30 seconds.
24. A method according to claim 1, further comprising a step of washing the silica magnetic particles after removal from the medium, before eluting the biological target material from the particles, using washing and elution buffers known *per se*.
- 25 25. A method according to any of the forgoing claims, wherein the method for isolating a biological target material from other material in a medium is an automated process.
- 30 26. Use of a method according to any of the forgoing claims for isolating a biological target material from other material in a medium.

27. Use of volume excluding agents to prevent clustering of magnetic particles in a fluidic medium.
28. Use according to claim 27, wherein the fluidic medium contains a biological target material and a salt.
29. A kit to perform a method according to claims 1 to 25, the kit comprising an aliquot of magnetic particles suspended in an aqueous solution in a first container and, optionally, other components needed to perform a method according to claims 1 to 25.
30. The kit of claim 29, wherein the magnetic particles are silica magnetic particles.
31. The kit of claim 30, wherein the silica magnetic particles are siliceous-oxide coated magnetic particles.

Abstract

The present Invention provides a method for isolating biological target materials, particularly nucleic acids, such as DNA or RNA or hybrid molecules of DNA and 5 RNA, from other substances in a medium using silica magnetic particles. The method of the present invention involves forming a complex of the silica magnetic particles and the biological target material in a mixture of the particles and the medium, comprising a salt and a volume excluding agent, separating the complex from the mixture using external magnetic force, and eluting the biological target material from 10 the complex, in a preferred embodiment in an automated process.

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